Green Synthesis of Nano-Particles using Bottle and Bitter Gourd and Study of its Antimicrobial Effects

RESEARCH PROTOCOL

For Registration for Ph.D. Programme

Doctor of Philosophy

By

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CERTIFICATE

This is to certify that this Research Proposal entitled "Green Synthesis of Nano-Particles using Bottle and Bitter Gourd and Study of its Antimicrobial Effects" by Anjali Bhardwaj submitted in fulfillment of the requirement for registration in Ph.D. program in Department of Biotechnology under Faculty of Sciences of JECRC University, Jaipur, during the academic year 2021, is a bonafide record of work carried out under my guidance and supervision.

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INTRODUCTION

Bionanotechnology has emerged up as integration between biotechnology and nanotechnology for developing biosynthetic and environmental friendly technology for synthesis of nanomaterials. (Shahcheraghi, et al., 2022). The size of nanomaterials is much smaller than that of most biological molecules and structures; therefore, nanomaterials can be useful in both in vivo and in vitro biomedical research application (Singh, et al., 2010).

Nanomedicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. A number of nanoparticles based therapeutics have been approved clinically for infections, vaccines, and renal diseases (Mitchell, et al., 2021).

Nanoparticles (NPs) can be synthesized using various approaches including chemical, physical, and biological approaches. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles and enhances its efficiency but chemicals used for this are toxic and lead to non-eco-friendly byproducts. (Dikshit, et al., 2021).The need for environmental nontoxic synthetic protocols for nanoparticles synthesis leads to the employment of biogenic route by utilizing aqueous plant extract and microbes, as they are considered as environment friendly, stable, bio-compatible, cost effective and clinically adaptable. Thus, there is an increasing demand for "Green Nanotechnology" in the field of nanoscience (Ahmed, et al., 2016; Mohanpuria, et al., 2008).

In green nanotechnology, many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi, and plants (Zhang, et al., 2020). Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals and provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganisms isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms (Garima, et al., 2011).

Nanotechnology is utilized for development in diverse fields such as biomedicines, catalysis, electrochemistry, food technology, pharmaceuticals, sensors, cosmetics, etc.(Bera and Belhaj, 2016; Frewer, et al., 2014; Dikshit, et al., 2021).

Nowadays, fabricated or modified of NPs are commonly used in industrially manufactured items e.g., cosmetics, textiles and electronics. The rapid increase in the number of microbes resistant to existing antibiotic drugs that has led to the requirement of novel medicines in the form of bare NPs. (Medina, et al., 2007; Deng, et al., 2016)

Based on physical and chemical characteristics, some of the important classes of nanoparticles are Carbon-based, Metal, Ceramic, Semiconductors, Polymers and Lipids. (Joudeh and Linke., 2022)
Metallic NPs (Au/Ag/Pd) are considered as a great source for biomedical applications which contain the most useful properties such as non-toxic, biocompatible, specific to target cells and tissues, highly stable aggregate activities, and very easily available.(Yaqoob, et al., 2020)

Silver Nanoparticles

Silver is an effective antimicrobial agent which exhibits low toxicity. The most important application of silver and silver nanoparticles (AgNPs) is in medical industry such as tropical ointments to prevent infection against burn and open wounds (Ahmad, et al., 2020). Silver nanoparticles (AgNPs) play a profound role in the field of biology and medicine due to their attractive physiochemical properties. Silver products have long been known to have strong inhibitory and bactericidal effects, as well as a broad spectrum of antimicrobial activities, which has been used for centuries to prevent and treat various diseases, most notably infections (Shankar, et al., 2005).

Silver nanoparticles are reported to possess antifungal, anti-inflammatory, antiviral, anti-angiogenesis, and antiplatelet activity (Sap-Lam, et al., 2010; Devi, et al., 2022)

Oligodynamic silver having antimicrobial efficacy extends well beyond its virotoxicity and it has lethal effects spanned across all microbial domains. The application of silver nanoparticles in drug delivery, drug discovery, and new drug therapies has declared war on many dreadful diseases and they use the body natural transport pathway and natural mechanism of uptake of the drug by the diseased cells (Prabhu, et al., 2010; Balaji., 2010).

Antibacterial activity of the silver containing materials is used in medicine to reduce infections in arthroplasty, as well as prevent bacteria colonization on prostheses, catheters, vascular grafts, dental materials, stainless steel materials, and human skin. Silver nanoparticles are widely used for its unique properties in catalysis, chemical sensing, biosensing, photonics, electronics, and pharmaceuticals. Silver nanoparticles have a great potential for use in biological including antimicrobial activity (Sarkar, et al., 2010).

As silver nanoparticles are showing a great enhance in the antimicrobial properties of plant extracts, we will prefer to use them in our further experiments.

REVIEW OF LITERATURE

Nanoparticles are tiny materials that consists of particulate substances of atleast less than 100nm of size. They are helpful in various fields, but they leave some harmful residues if synthesized by using chemicals. If plants or rather say plant extracts are used for nanoparticle synthesis, they do wonders. Biosynthesized nanoparticles are gaining popularity because of biologically active secondary metabolites of plants that help in green synthesis and also due to their unique biological applications (Hano and Abbasi., 2022).

Previous studies reports a reliable, ecofriendly and cost-effective synthesis of silver nanoparticles using the aqueous plant extracts and their antibacterial and antiproliferative activity (Hemlata, et al., 2020).

Silver nanoparticles can be biosynthesized using the aqueous plant extracts which acted as a reducing and capping agent. Various parts of plant such as leaves, peel, seed, stem, roots, flowers, fruits, rhizomes *etc.*, have been successfully utilized for the synthesis of AgNPs. The synthesis of NPs is completely dependent on the biomolecules/phytochemicals present in the plant extract. (Sumitha, et al., 2018).

Few related studies are reviewed below for a better understanding of the previous work and getting an idea about what objectives we can take for further research work.

Clitoria ternatea and *Solanum nigrum* were reported to synthesize very small-size AgNPs and evaluated against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella aerogenes*. Out of these two, leaf *Clitoria ternatea* extract gave smaller nanoparticles, which indicated the important role of extract constituents on the size of the produced nanoparticles. In addition, AgNPs of *Clitoria ternatea* showed higher activity than the AgNPs of *Solanum nigrum* against nosocomial pathogens because to its small size. It has been well-documented in literature that smaller size AgNPs showed higher antimicrobial activities due to larger surface area (Krithiga, et al.,2015).

Pimpinella anisum (Alsalhi, et al., 2016), *Synsepalum dulcificum* (Lateef, et al., 2016), *Vigna radiata* (Choudhary, et al., 2016), *Dracocephalum moldavica* (Pak, et al., 2016) leaf extracts were also successfully applied for the green synthesis of AgNPs. *Vigna radiata* mediated AgNPs, was found to

be more susceptible towards Gram-negative bacteria *E. coli* (ZOI 20 mm) than Gram-positive *S. aureus* (ZOI 16 mm) due to the higher thickness of the peptidoglycan layer (approx. 80 nm thick) of the cell wall of Gram positive bacteria which is 10 times thicker than the peptidoglycan Gram-negative bacteria, hence is less susceptible to be destroyed by AgNPs (Choudhary, et al., 2016).

In a research, the biosynthesized *Cucumis prophetarum* silver nanoparticles (Cp-AgNPs) were characterized using different techniques, such as UV–visible spectroscopy, dynamic light scattering (DLS), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDAX). Phytochemical analysis was performed to determine the phytochemicals responsible for the reduction and capping of the biosynthesized Cp-AgNPs. The antioxidant activity of the biosynthesized nanoparticles was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. Their antibacterial activity was checked against *Staphylococcus aureus* (Gram-positive) and *Salmonella typhi* (Gram-negative) bacteria. The biosynthesized nanoparticles showed dosage-dependent inhibition activity with a significant zone of inhibition (ZOI) and were more effective toward *S. typhi* as compared to *S. aureus*. This work revealed that the biosynthesized silver nanoparticles using *C. prophetarum* leaf extract were associated with good antibacterial activity and antiproliferative potential against selected cancer cell lines. The biosynthesized *C. prophetarum* AgNPs can be further exploited as a potential candidate for antioxidant, antibacterial, and anticancer agents (Hemlata, et al., 2020).

Recently,use of peel extract as a reducing agent for the green synthesis of nanoparticles with selective size gaining immense attention. Till now, various peel extract was used for the biosynthesis of AgNPs. Peel extract of *Cavendish banana* (kokila, et al., 2015), banana (*Musa paradisiaca*) (Ibrahim., 2015), *Carica papaya* (Balavijayalakshmi and Ramalakshmi., 2017) and *Citrus sinensis* were utilized for the green synthesis of AgNPs. The synthesized AgNPs were tested against various bacterial strains to investigate its bactericidal activity and found that the AgNPs obtained from various peel extract displayed great cell disruption. The influence of various factors on the bio-reduction of $AgNO_3$ was investigated and found that 1.75 mM $AgNO_3$, 20.4 mg dry banana peel 4.5 pH and 72 hr incubation time. The authors reported that the synthesized AgNPs displayed synergistic effects with the antibiotic levofloxacin (Ibrahim., 2015). *Citrus maxima* peel extract was utilized as both reducing and capping agent for the green synthesis of AgNPs with size ranging from 4–11 nm. Bactericidal activity of the synthesized AgNPs was tested against *E. coli* and *S. aureus* and found that the *Citrus maxima* peel extract mediated AgNPs showed great inhibitory action against both the bacterial strain. Also, bactericidal activity was examined against plant pathogens such as *Fusarium*

oxysporum and *Verticillium dahliae*, showed excellent inhibitory action against both the pathogens (Huo, et al., 2018). Besides, *Punica granatum* (Annu, et al., 2018), *Citrus × clementine*, (Saratale, et al., 2017) and *Solanum melongena* L.(Das, *et al.,* 2019) peel extracts were exploited for the green synthesis of AgNPs and tested its bactericidal activity against various bacterial strains. The results revealed that the synthesized AgNPs displayed great cell wall damage of both Gram-positive and Gram-negative bacteria. Interestingly, AgNPs derived from *Punica granatum* peel extract showed high ZOI against *S. aureus* (16.5 mm) compared to *E. coli* (15.5 mm) (Annu , et al., 2018). Microwave irradiation technique has been utilized for the green synthesis of AgNPs from *Solanum melongena* L. peel extract to increase the rate of bioreduction process of $AgNO_3$. Moreover, the size and the shape of the nanoparticles generated *via* this process is 92.4 nm and spherical (Das, et al., 2019). In another work, Dutta, et al., 2020 have reported the green synthesis of AgNPs using *Citrus limetta* peel extract as both reducing and capping agent. Investigation of bactericidal activity of the synthesized AgNPs against various pathogens such as *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Micrococcus luteus*, *Streptococcus mutans*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Eschrechia coli* revealed that the *Citrus limetta* peel extract mediated AgNPs have cell disruption potential and hence can be used in pharmaceutical industries. Further, the antifungal activity test of the synthesized AgNPs against *Candida* species revealed that the nanoparticle has the ability of cell membrane distortion. The effect of AgNPs on micro-morphological changes of *C. albicans* was clearly visible and found that AgNPs induces the cell blebs and a thick exudate deposition around the cell that demonstrate the leakage of intercellular components. From the results, the authors reported that *Citrus limetta* peel extract mediated AgNPs have excellent antifungal activity.

Plant seed extract also well established for the biosynthesis of nanoparticles. Till now, various seeds extract has been utilized for the biosynthesis AgNPs. *Sinapis arvensis* seeds mediated AgNPs was reported for more than 83% inhibition of mycelium growth of fungus *Neofusicoccum parvum*. Inductively coupled plasma (ICP) spectrometry analysis revealed complete reduction of Ag^+ to Ag^0 in more than 95% conversion within 50 days of reaction (Khatami , et al., 2015).

In another work, grape seed extract was utilized for the biosynthesis of spherical and polygonal AgNPs with size ranging from 25–35nm. Bactericidal activity of the synthesized NPs was tested against eight different ocean pathogenic bacteria and it showed great inhibition activity only against four bacteria such as *Vibrio alginolyticus*, *Vibrio anguillarum* and *Vibrio parahaemolyticus* (Xu, et al., 2015). Sumitha *et al.* reported bio-reduction of $AgNO_3$ to AgNPs using *Durio zibethinus* seed extract. It is reported that saccharides present in the extract induces the bio-reduction and the amino acids present in the extract stabilized the synthesized AgNPs. Bactericidal activity was tested against

different pathogenic bacteria and found that the NPs showed greater activity against *S. typhimurium, S. haemolyticus* and *S. aureus* over *B. subtilis, E. coli* and *S. typhi*.

Few literatures investigated that the efficiency of AgNPs as antimicrobial agent is extensively dependent on the shape of the nanoparticles. The comparison of spherical, disc like and triangular shaped AgNPs as antimicrobial agent revealed the activity trend follows as spherical AgNPs > disc-like AgNPs > triangular AgNPs. (Salar, et al., 2015). The highest inhibition effect of 94.1% and 84% was observed at 40 ppm concentration of AgNPs against *R. solani* and *N. parvum* respectively, using AgNPs derived from *Trifolium resupinatum* seeds extract (Khatami, et al., 2016). In a similar study, Khatami., et al. reported more than 86% inhibition of mycelium growth of *R. solani* at a concentration 25 μg mL^{-1} (or 25 ppm) of the biogenic AgNPs (Khatami , et al., 2016). Several plant seeds such as *Nigella arvensis, Linseed* (Haseeb, et al., 2017), *Embelia ribes* (Dhayalan, et al., 2016), *Melissa officinalis* (Pirtarighat, et al., 2017), are applied for the generation of spherical shape AgNPs. While biogenic AgNPs are reported to be more efficient antimicrobial than any other metal NPs in most of the experiments, it is worth mentioned that the *Embelia ribes* derived AgNPs is less susceptible to *E. coli* at showing ZOI of 20 mm against 28 mm ZOI for gold nanoparticles (AuNPs) at 250 μL mL^{-1} concentration (Dhayalan, et al., 2016). Although having small size of NPs (6–25 nm), *Leucaena leucocephala* mediated AgNPs displayed very low toxicity against both *E. coli* and *S. aureus* with ZOI of 18 mm and 22 mm (approx.) respectively at 1000 ppm AgNPs concentration (Kumar, et al., 2017). *Alpinia katsumadai* seeds extract mediated AgNPs showed excellent activities against *E. coli* and *S. aureus* than that of *P. aeruginosa* (He, et al., 2017), whereas those derived from *Myristica fragrans* are found to be highly sensitive to multidrug-resistant (MDR) *Salmonella enterica serovar typhi* (*S. typhi*) where a highest ZOI of 16.4 ± 0.45 was observed at 100 μg μL^{-1} concentration of AgNPs (Balakrishnan, et al., 2017).

The seeds extracts of plants such as *Tectona grandis* (Rautela, et al., 2019), *Persea americana* (Girón-Vázquez, et al., 2019), *Salvia hispanica* and *Trigonella foenum-graecum* (Varghese, et al., 2019) produced AgNPs with high antimicrobial activities. Interestingly the size of AgNPs depends on the concentration of *Persea americana* extract where a small NPs was recorded at low concentration of aqueous extract, whereas high concentration results in the formation of larger NPs (Girón-Vázquez, et al., 2019). Ironically, the AgNPs from *Salvia hispanica* L showed lower susceptibility towards antibiotic Ampicillin against *E. coli* and *S. aureus* although its high ZOI against *E. coli* (18.5 mm) and *S. aureus* (14.9 mm) at 7.7 μL mL^{-1} concentration.

Fruit mesocarp of *Lagenaria siceraria* (Molina) Standl. also reported for the synthesis of AgNPs using it's methanolic extract and evaluated against several microbes. The antimicrobial assays were conducted against bacterial strains including *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and fungal strain including *Candida albicans* by agar well diffusion method. This study revealed that methanolic extract of *L.siceraria* fruit mesocarp and its various fractions possessed antimicrobial potential of varying degree, suggests their application for defense against different microbes in form of nanoparticles (Dar, et al., 2017).

Another investigation demonstrates the formation of silver nanoparticles by the reduction of the aqueous silver metal ions during exposure to the *Lagenaria siceraria* leaf extract. Inhibitory activity of AgNPs and leaf extract were tested against human pathogens like gram-positive (*Staphylococcus aureus, Bacillus subtilis*), gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*). The observations indicated that the AgNPs showed less inhibitory actions against human pathogens than *Lagenaria siceraria* leaf extract, showing its antimicrobial value against pathogenic diseases.(Anandh, et al., 2014)

Recently, antioxidant efficacy and antibacterial activity of green synthesis of AgNPs using *Momordica charantia* L. extract was studied. For antioxidant ability for the Bitter guard-AgNPs, the Aqueous BG-AgNPs displayed a higher degree of antioxidant performance as compared to Ethanolic BG-AgNPs, due to the greater abundance of phenolic compounds that existed in the aqueous BG extracts and AgNPs. The MIC(Minimum Inhibitory Concentration) tests revealed that both BG-AgNPs showed limited hemolysis of RBCs and excellent antibacterial activity against non- and drug-resistant bacterial strains (Moorthy. *Et al.,* 2021).

According to review literature, less research work has been done on the peel and seed extract of *L. siceraria* and *M. charantia* and their antimicrobial activity with various pathogenic microbes. So we are taking these two members of cucurbitaceae for our further research work and will try to enhance the efficacy of silver nanoparticles to encounter the impact of pathogenic microbes.

OBJECTIVES OF THE STUDY

- To obtain the seed and peel extract of the *Lagenaria siceraria* and *Momordica charantia*
- To form the silver nanoparticles of the sample extracts
- To test the antimicrobial properties of the silver nanoparticles.

MATERIALS

Plant Materials

The Cucurbitaceae, also called cucurbits or the gourd family, are a plant family consisting of about 965 species in around 95 genera, of which the most important to humans are: *Cucurbita ,Lagenaria ,Citrullus ,Cucumis ,Momordica ,Luffa*.

The family Cucurbitaceae ranks among the highest of plant families for number and percentage of species used as human food.The name Cucurbitaceae comes to international scientific vocabulary from New Latin, from Cucurbita, the type genus, + -aceae, a standardized suffix for plant family names in modern taxonomy. The genus name comes from the Classical Latin word cucurbita, "gourd".Members of this family are mesophytic or xerophytic, mainly tropical or subtropical. They are equally distributed in the eastern and western hemispheres . Cucurbits are annual, or perennial with tuberous storage roots and frost sensitive, a factor that limits their geographic distribution and area of cultivation. (Chomicki, et al.,2020)

The stems are hairy and pentangular. Tendrils are present at 90° to the leaf petioles at nodes. Leaves are exstipulate, alternate, simple palmately lobed or palmately compound. The flowers are unisexual, with male and female flowers on different plants (dioecious) or on the same plant (monoecious).(Deore, 2020)

The typical pollinators are bees and moths. Less often, the rich nectar supply and pollen within the plant draws hummingbirds and bats. The UV-reflective petals direct the pollinators. The female flowers contain gynoecium that mimics the structure of the androecium in the male flowers and can fool insects.

Lagenaria siceraria, also known as bottle gourd, is a vine grown for its fruit. It can be either harvested young to be consumed as a vegetable, or harvested mature to be dried and used as a utensil, container, or a musical instrument. When it is fresh, the fruit has a light green smooth skin and white flesh. (Anandh, et al., 2014)

kingdom: Plantae
Order: Cucurbitales
Family: Cucurbitaceae
Genus: *Lagenaria*
Species: *siceraria* (Molina) Standl.

Momordica charantia, a member of the Cucurbitaceae family, is also known as bitter melon, bitter gourd, balsam pear, pare, or karela. It is a widely grown and consumed vegetable in Asia, East Africa, India, and South America. The potential for MC to modulate blood glucose has received the most attention from investigators searching for natural foods or compounds that may be useful in the treatment of diabetes. (Moorthy. et al., 2021).

Kingdom: Plantae

Order: Cucurbitales

Family: Cucurbitaceae

Genus: *Momordica*

Species: *charantia* L.

METHODOLOGY TO BE ADOPTED

1.Identification,Collection and Preparation of Plant Materials

Fresh seeds and peel of two different plants, that is, *Lagenaria siceraria* and *Momordica charantia*, free from diseases will be collected and then washed thoroughly 2-3 times with tap water followed by distilled water to exclude debris and any other unwanted materials.

After that, the portions will be dried and ground separately to make powder. Further the powders of seed and peel of *L.siceraria* and *M.charantia* will be boiled in distilled water about 30-45 mins, below 60 °C as high temperature heating for long time may leads to the decomposition of phytochemicals in the biomass extract (Ali ,et al., 2015). Mixture will be cooled and filtered through Whatman No.1 filter paper (pore size 125mm) and then the filterate will be collected and stored at 4 °C before use.(Ruman and Kia., 2021)

2. Synthesis of Silver Nanoparticles

The Erlenmeyer flask containing 50 mL of L. siceraria and M. charantia extracts of seed and peel will be mixed separately with 0.05 m/moL of AgNO3 under continuous stirring at 30 °C. The color of solutions will be changed from light brown into dark brown color after several min which indicates formation of silver nanoparticle. The dark brown solid products will be collected through centrifugation at 4000 rpm for 15 min. After washing several times with distilled water, the obtained solid products will be dried in an oven at 60°C. The final powders will be stored in airtight bottles for further analysis.(Ruman and Kia., 2021)

This AgNPs synthesis process avoids the use of chemical stabilizer as biomaterials present in the extract act as a reducing agent as well as a stabilizing agent for the synthesis of AgNPs. (Manikandan, et al., 2015; Biswas, et al., 2018)

The progress of the formation of AgNPs can be monitored by visual color changes or using UV-Vis. Spectroscopy, where a sharp peak due to surface plasmon resonance (SPR) of AgNPs at around 430–450 nm is clearly observed.(Vanlalveni, et al., 2018)

3. UV-Visible Absorbance Spectroscopy

UV-Visible spectroscopy analysis will be carried out on a Systronic UV Visible absorption spectrophotometer 117 with a resolution of ±1 nm between 200 and 1000 nm processing a scanning speed of 200 nm/min.

Equal amounts of the suspension (0.5 mL) will be taken and analysed at room temperature.

The progress of the reaction between metal ions and the plant extract will be monitored by UV-Visible spectra of silver nanoparticles in aqueous solution with different wavelength in nanometers from 340 to 800 nm. The reduction of silver ions and formation of silver nanoparticles will occur within an hour of reaction. Control will be maintained by using AgNO3 (Shankar, et al., 2005).

4. Fourier Transforms Infrared Spectroscopy (FTIR)

For FTIR measurements, the synthesized silver nanoparticles solution will be centrifuged at 10000 rpm for 30 minutes.

The pellet will be washed thrice with 5 mL of deionised water to get rid of the free proteins or enzymes that are not capping the silver nanoparticles.

The pellet will be dried by using vacuum drier and analysed by FTIR. (Prema, 2010.)

5. X-Ray Diffraction

A thin film of the silver nanoparticle will be made by dipping a glass plate in a solution and carried out for X-ray diffraction studies.

The crystalline silver nanoparticle will be calculated from the width of the XRD peaks. (Prema, 2010.)

6. Scanning Electron Microscopy

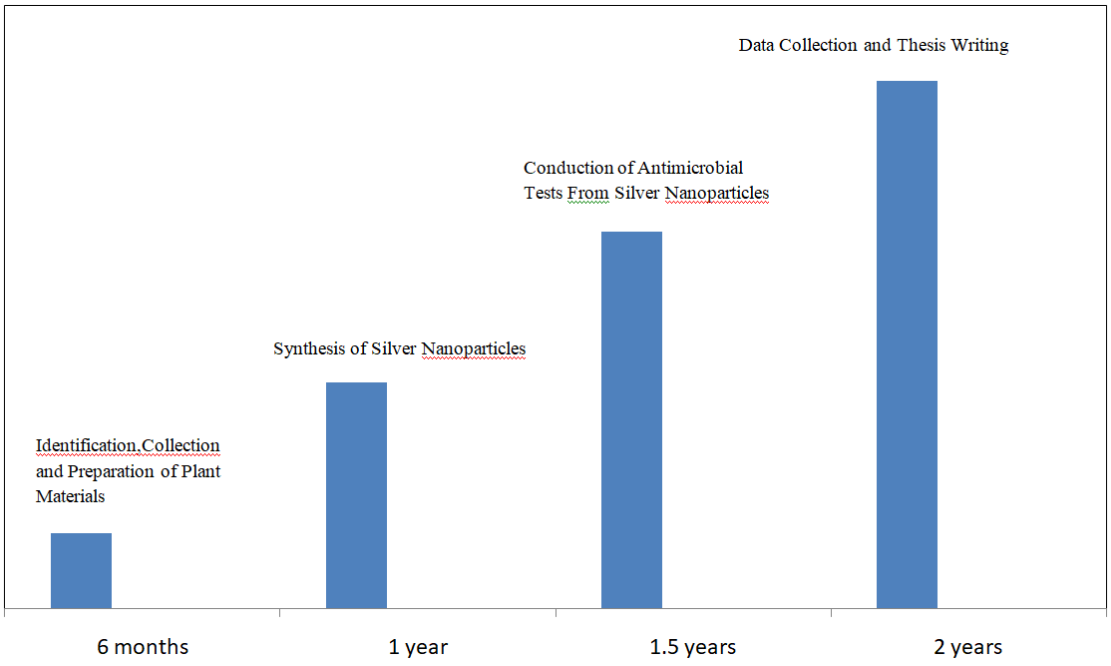
The pellet will be subjected for SEM analysis. Thin films of the sample will be prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid; extra solution will be removed using a blotting paper and then the film on the SEM grid will be allowed to dry under mercury lamp (5 mins) for analysis. (Sukhwal, et al., 2017)

7. Antibacterial Assays

The antibacterial assays will be done with pathogens by standard disc diffusion method. Briefly Nutrient agar medium will be used to cultivate bacteria.

Fresh overnight culture of inoculum (100 μL) of each culture will be spread on to Nutrient Agar (NA) plates. Sterile paper disc of 5 mm diameter containing 10μg/mL silver nanoparticles along with control (Plant Extract) and Silver nitrate, will be placed in the nutrient agar plates. The plates will be incubated at 37°C for 24hrs. Next day the inhibition zones around the discs will be measured. The experiments will be repeated thrice and mean values of ZOI will be considered as final value. (Rao and Savithramma, 2012)

TIMELINE


Data Collection and Thesis Writing
Conduction of Antimicrobial Tests From Silver Nanoparticles
Synthesis of Silver Nanoparticles
Identification,Collection and Preparation of Plant Materials
6 months
1 year
1.5 years
2 years

PROPOSED OUTCOMES OF THE RESEARCH

The reduction of silver nitrate using the seed and peel extract will be viewed by the colour change in the reaction solutions .

In UV-V spectra will be recorded for the reaction solution of reduced silver nitrate by seed and peel extract of *Lagenaria siceraria* and *Momordica charantia.*

The maximum absorbance peak will be obtained for *Lagenaria siceraria* and *Momordica charantia* respectively.

FTIR measurements will be carried out to identify the possible biomolecules responsible for the capping and efficient stabilization of the silver nanoparticles synthesized by the plant extracts.

Inhibition zones will be measured for different microbial species by nanoparticles of plant extracts.

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